Validation of the Spanish-language version of the Rapid Assessment for Adolescent Preventive Services among Colombian adolescents

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ABSTRACT
Seventy percent of adolescent morbidity and mortality is related to six risky behaviors.
The Rapid Assessment for Adolescent Preventive Services is a screening questionnaire consisting of 21 questions but there is not a validated Spanish-language version.
The objective of this study was to validate the Spanish-language version of the Rapid Assessment for Adolescent Preventive Services in two Colombian cities: Bucaramanga and Medellín. The questionnaire was administered to 270 randomly selected adolescent students aged between 11 and 19 years old. Its internal consistency measured using Cronbach's alpha was 0.7207. The factor analysis showed that two factors accounted for 84.5% of variance, but factor loading indicates that only one of these is valid in Colombia: substance use (tobacco, alcohol, narcotics, and psychoactive substances).
Key words: *adolescent, result validity, Rapid Assessment for Adolescent Preventive Services, dangerous behavior.*

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INTRODUCTION

Adolescence is a critical stage in the life cycle[1] that ends in personality and identity formation.[2] In the United States, up to 70% of adolescent morbidity and mortality is related to risky behaviors, including injuries, alcohol or psychoactive substance use, risky sexual behavior, tobacco use, unhealthy eating habits, and physical inactivity.[3,4] An early identification of these behaviors allows to provide adequate support and develop prevention strategies.[5] However, lack of time during office visits becomes a great hurdle in their identification. For this reason, several screening instruments have been developed that aim at being time-effective. The Rapid Assessment for Adolescent Preventive Services (RAAPS) is one of these instruments.

The RAAPS was developed by the University of Michigan's School of Nursing. It consists of 21 questions related to six risky behaviors.[6] Each risky behavior accounts for a domain in the theoretical construct which serves as the basis for this scale. The validity index of the English-language version is 0.825-1.0, and its inter-rater kappa is 0.44-0.99, granting the construct a highly relevant validity.[7]

There is a Spanish-language version of the RAAPS which has not been validated. This assessment may turn into a useful tool in primary care settings catering for Spanish-speaking adolescents.

The objective of this study is to validate the Spanish-language version of the RAAPS to screen for risky behaviors among Colombian adolescents.

MATERIALS AND METHODS

A study was conducted to validate the construct of the RAAPS Spanish-language version. High school students aged between 11 and 19 years old living in Bucaramanga and Medellín, with no disorders of perception that would prevent them from reading and writing with pen and paper, were included.

Permission to use the test was requested to its authors, who supplied the Spanish-language version provided that it was administered unchanged (*Table 1*). Participants referred comprehension problems with certain parts of the text, which were recorded and reported to authors after its validation. This study was approved by the Research Ethics Committee of Universidad Industrial de Santander (Resolution no. 30, December 5th, 2014).

Based on 80% reliability, it was expected that at least 10 subjects by item would be detected, in addition to a 10% potential loss due to parental refusal to participate. This meant that at least 230 adolescents had to be included.

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Participants were selected using a cluster random sampling process, which allowed identifying all high schools and the number of courses in each school in 2014; 18 groups from both cities were selected. Every student in each group was invited to participate. They were given an informed consent form to be duly completed by their parents or tutors and an assent form for themselves.[8] Students who agreed to participate, and had both forms signed, completed the RAAPS anonymously, with no time constraints, supervision or help to answer any of the items.

Questionnaires that did not indicate the participant's age were excluded from the analysis. Although the RAAPS was designed for 11 to 19-year-old adolescents, we decided to include students who were 10 years old at the time of the survey because they were already in high school and would turn 11 during the school year. They were also considered to be exposed to the same risk factors.

The prevalence of affirmative answers to each of the 21 items was estimated together with a 95% confidence interval (95% CI), and data were adjusted given the sampling process used, which may differ from the crude estimation of affirmative answers/population.[9] For item 16, the proportion was estimated based on the total number of students who indicated to have had sexual intercourse.

Construct validity was analyzed with the Stata 12.1 software (StataCorp., College Station, USA, 2014) using Cronbach's alpha (overall and by item) and factor analysis to verify how theoretical

TABLE 1. *Spanish-language version of the Rapid Assessment for Adolescent Preventive Services*

1. In the past 12 months, have you tried to lose weight by obsessively exercising, taking diet pills or laxatives, making yourself vomit (throw up) after eating, or starving yourself?
2. Do you eat some fruits and vegetables every day?
3. Are you active after school or on weekends (walking, running, dancing, swimming, biking, playing sports) for at least 1 hour, on at least 3 or more days each week?
4. Do you always wear a seat belt when you are driving or riding in a car, truck, or van?
5. Do you always wear a helmet when you are biking, rollerblading, skateboarding, motorcycling, snowmobiling, skiing or snowboarding?
6. During the past month, have you been threatened, teased, or hurt by someone (on the internet, by text, or in person) or has anyone made you feel sad, unsafe, or afraid?
7. Has anyone ever abused you physically (hit, slapped, kicked), emotionally (threatened or made you feel afraid) or forced you to have sex or be involved in sexual activities when you didn't want to?
8. Have you ever carried a weapon (gun, knife, club, other) to protect yourself?
9. In the past 3 months, have you smoked cigarettes or any other form of tobacco (cigars, black and mild, hookah, other) or chewed/used smokeless tobacco?
10. In the past 12 months, have you driven a car drunk, high, or while texting or ridden in a car with a driver who was?
11. In the past 3 months, have you drunk more than a few sips of alcohol (beer, wine coolers, liquor, other)?
12. In the past 3 months, have you smoked marijuana, used other street drugs, steroids, or used inhalants ("huffed" household products)?
13. In the past 3 months, have you used someone else's prescription (from a doctor or other health provider) or any non-prescription (from a store) drugs to sleep, stay awake, concentrate, calm down, or get high?
14. Have you ever had any type of sex (vaginal, anal or oral sex)?
15. Have you ever been attracted to the same sex (girl to girl/guy to guy) or do you feel that you are gay, lesbian, or bisexual?
16. If you have had sex, do you always use a method to prevent sexually transmitted infections and pregnancy (condoms, female barriers, other)?
17. During the past month, did you often feel sad or down as though you had nothing to look forward to?
18. Do you have any serious problems or worries at home or at school?
19. In the past 12 months, have you seriously thought about killing yourself, tried to kill yourself, or have you purposely cut, burned or otherwise hurt yourself?
20. Do you have at least one adult in your life that you can talk to about any problems or worries?
21. When you are angry, do you do things that get you in trouble?

domains behaved, at first directly and, if the model was inadequate, making rotations. Factors were considered likely if they had an eigenvalue >1.0 while items were considered part of a given factor if their factor loading was ≥0.5.[10,11]

RESULTS

Out of 275 questionnaires administered between March and July 2015, five were excluded because participants had not indicated their age. Among those completed, 65.9% corresponded

TABLE 2. *Prevalence of affirmative answers among 270 participating students*

Question	n (Crude prevalence)	Adjusted prevalence (95% CI)
Tries to lose weight inadequately (12 months)	21 (7.8%)	8.6% (5.2-12.0)
Eats fruits and vegetables every day	186 (68.9%)	74.8% (69.5-80.1)
Is physically active outside school	186 (68.9%)	74.3% (69.0-79.5)
Always wears a seat belt	159 (58.9%)	61.9% (56.1-67.8)
Always wears a helmet	121 (44.8%)	47.4% (41.4-53.4)
Has been a victim of bullying (past month)	44 (16.3%)	16.7% (12.2-21.1)
Has been abused physically, emotionally or sexually	24 (8.9%)	8.9% (5.5-12.3)
Has carried a weapon	23 (8.5%)	10.0% (6.4-13.7)
Has smoked tobacco (3 months)	12 (4.4%)	4.8% (2.2-8.2)
Has driven a car drunk high, or while texting (12 months)	13 (4.8%)	5.6% (2.8-8.3)
Has drunk more than a few sips of alcohol (3 months)	57 (21.1%)	23.7% (18.6-28.8)
Has abused psychoactive substances or steroids (3 months)	8 (2.9%)	2.4% (1.9-2.9)
Has abused medications (12 months)	11 (4.1%)	3.3% (1.1-5.5)
Has had any type of sex	34 (12.6%)	12.8% (8.8-16.9)
Is attracted to the same sex or feels that he/she is gay, lesbian, or bisexual	12 (4.4%)	4.5% (2.0-7.0)
Always uses methods to prevent STI/pregnancy*	24 (70.5%)	79.4% (62.1-91.3)
Has felt depressed (1 month)	64 (23.7%)	27.1% (21.7-32.4)
Has had serious problems at home/school	51 (18.9%)	20.0% (15.2-24.8)
Suicide/self-harm thoughts/attempts (12 months)	23 (8.5%)	10.2% (6.5-13.8)
Has one adult that he/she can talk to about any worries	201 (74.4%)	80.5% (75.7-85.3)
When angry, does things that get him/her in trouble	75 (27.7%)	31.5% (25.9-37.1)

* Proportion estimated among the 34 students who indicated to have had sex.

TABLE 3. *Cronbach's alpha analysis of the Rapid Assessment for Adolescent Preventive Services*

Ítem	Inter-item correlation	Item-test correlation	Item's alpha
Tries to lose weight inadequately (12 months)	0.3322	0.2345	0.7132
Eats fruits and vegetables every day	0.2606	0.2606	0.7137
Is physically active outside school	0.0828	0.0828	0.7322
Always wears a seat belt	0.2119	0.2119	0.7207
Always wears a helmet	0.2640	0.2640	0.7155
Has been a victim of bullying (past month)	0.3634	0.3634	0.7033
Has been abused physically, emotionally or sexually	0.2891	0.2891	0.7209
Has carried a weapon	0.3625	0.3625	0.7051
Has smoked tobacco (3 months)	0.3997	0.3997	0.7065
Has driven a car drunk, high, or while texting (12 months)	0.0410	0.0410	0.7255
Has drunk more than a few sips of alcohol (3 months)	0.3992	0.3992	0.6982
Has abused psychoactive substances or steroids (3 months)	0.3518	0.3518	0.7108
Has abused medications (12 months)	0.2839	0.2839	0.7130
Has had any type of sex	0.1990	0.1990	0.7171
Is attracted to the same sex or feels that he/she is gay, lesbian, or bisexual	0.3839	0.3839	0.7077
Has felt depressed (1 month)	0.4421	0.4421	0.6934
Has had serious problems at home/school	0.3565	0.3565	0.7036
Suicide/self-harm thoughts/attempts (12 months)	0.4433	0.4433	0.6992
Has one adult that he/she can talk to about any worries	0.3192	0.3192	0.7073
When angry, does things that get him/her in trouble	0.3089	0.3089	0.7089

to Bucaramanga, 91.1% to students attending public schools, 56.1% were boys, and 81.1% lived in a middle socioeconomic level area. Students' mean age was 13.6 years old (standard deviation: 1.8 years old).

Table 2 shows the crude and adjusted prevalence of affirmative answers to the RAAPS items.

Cronbach's alpha was 0.7207 and ranged between 0.6982 and 0.7322 by item (*Table 3*). Factor analysis did not include item 16 because it was answered only by students who had had sexual intercourse. Two factors were thus observed: a factor with a 2.969 eigenvalue, which accounted for 61.44% of variance, made up of three items related to alcohol, psychoactive substance, and tobacco use. The second factor (with an eigenvalue of 1.116) accounted for 23.09% of variance, but there were no prevalent items. After making rotations, factor resolution did not improve (*Table 4*).

DISCUSSION

This is the first validation of the Spanish-language version of the RAAPS. Construct validity was acceptable, with no remarkable modifications after removing any of the items.[10] However, only one factor made up of three items related to substance use was identified,[11] and this indicates that, in Colombia, the RAAPS may only result useful to assess these risk factors.

Using factor analysis to assess constructs is based on verifying agreement or lack of agreement between the items exploring a risky behavior or domain and the answers given by a population.[11] This means that, if the theoretical construct was true, questions specific to each risky behavior should be solved in the same direction and be part of the same factor. Thus, the North American theoretical risky behavior construct used to develop the RAAPS does not fit the Colombian setting.

Most of the participants attended public schools. In 2014, 82.9% of students in Bucaramanga and Medellín were enrolled in public schools, so the fact that the average number of students per course in public schools was twice the number in private schools does not allow to infer a bias in the cluster random sampling process.

The fact that the six expected risky behaviors did not appear in the factor analysis indicates

TABLE 4. *Observed factor loading*

Aspect	Factor 1	Factor 2	Factor 3
Eigenvalue	2.969	1.116	0.748
Explained variance	61.44%	23.09%	15.49%
Item			
Tries to lose weight inadequately (12 months)	0.279	-0.168	-0.092
Eats fruits and vegetables every day	-0.218	0.073	0.066
Is physically active outside school	-0.036	0.214	-0.043
Always wears a seat belt	-0.156	0.084	0.356
Always wears a helmet	-0.213	0.055	0.358
Has been a victim of bullying (past month)	0.463	-0.329	0.263
Has been abused physically, emotionally or sexually	0.320	-0.314	0.279
Has carried a weapon	0.412	-0.020	-0.170
Has smoked tobacco (3 months)	0.620	0.218	0.106
Has driven a car drunk, high, or while texting (12 months)	0.064	0.269	-0.188
Has drunk more than a few sips of alcohol (3 months)	0.498	0.063	-0.253
Has abused psychoactive substances or steroids (3 months)	0.652	0.469	0.158
Has abused medications (12 months)	0.315	-0.149	-0.156
Has had any type of sex	0.284	0.311	-0.290
Is attracted to the same sex or feels that he/she is gay, lesbian, or bisexual	0.480	-0.128	0.071
Has felt depressed (1 month)	0.455	-0.396	-0.047
Has had serious problems at home/school	0.433	-0.176	0.108
Suicide/self-harm thoughts/attempts (12 months)	0.461	-0.313	-0.170
Has one adult that he/she can talk to about any worries	-0.280	0.216	0.049
When angry, does things that get him/her in trouble	0.360	-0.092	-0.092

that construct validity is not a property of the test itself, but of the population where it is administered.[12] The RAAPS was designed for North American students but, in this study, it was assessed in Colombian students, among whom different behaviors may prevail, especially considering that half of the participants were younger than 15 years old. It is also possible that participants were not even aware of the information necessary to answer regarding some behaviors, such as using a seat belt or the type of food they eat. However, the similar prevalence of affirmative answers regarding items related to alcohol, tobacco and illegal substance use, sexual intercourse initiation,[14] and depression prevalence[15] renders this explanation less likely.

Finally, the small number of questions per domain also hinders the possibility of achieving factor loading for at least three of them to be considered relevant for factor analysis.

Also, the effect on the lack of detection of other risky behavior groups cannot be estimated because the test has not been transculturally adapted; so it is necessary to study the RAAPS validity in depth and explore potential differences in behavior by outcome measure, e.g., age or sex groups, especially among adolescents older than 16 years old, in addition to assessing intra-subject reproducibility and sensitivity to change.

CONCLUSION

The RAAPS construct validity in the Colombian setting is acceptable but limited. It is very useful to detect alcohol, psychoactive substance, and tobacco use. ■

REFERENCES

1. OMS. Salud para los adolescentes del mundo: una segunda oportunidad en la segunda década. Ginebra: Servicio de Producción de Documentos de la OMS, 2014. [Accessed on: March 3, 2016]. Available at: http://apps.who.int/adolescent/second-decade/files/ WHO_FWC_MCA_14.05_spa.pdf.
2. UNICEF. Estado Mundial de la Infancia 2011: La adolescencia una época de oportunidades. Nueva York, 2011. [Accessed on: March 3, 2016]. Available at: https://web.oas.org/childhood/ES/Lists/Recursos%20%20Bibliografia/Attachments/17/5.pdf.
3. Eaton DK, Kann L, Kinchen S, Shanklin S, et al. Youth risk behavior surveillance - United States, 2009. MMWR Surveill Summ 2010;59(5):1-142.
4. Shanklin S, Brener ND, Kann L, Griffin-Blake S, et al. Youth risk behavior surveillance--selected steps communities, United States, 2007. MMWR Surveill Summ 2008;57(12): 1-27.
5. Bradford S, Rickwood D. Psychosocial assessments for young people: a systematic review examining acceptability, disclosure and engagement, and predictive utility. Adolesc Health Med Ther 2012;3:111-25.
6. Yi CH, Martyn K, Salerno J, Darling-Fischer CS. Development and clinical use of Rapid Assessment for Adolescent Preventive Services (RAAPS) questionnaire in school-based health centers. J Pediatr Health Care 2009; 23(1):2-9.
7. Salerno J, Marshall VD, Picken EB. Validity and reliability of the rapid assessment for adolescent preventive services adolescent health risk assessment. J Adolesc Health 2012;50(6):595-9.
8. República de Colombia. Ministerio de Salud. Resolución N.° 008430 de 1993. Bogotá, 4 de octubre de 1993. [Accessed on: March 3, 2016]. Available at: https://www.invima.gov.co/images/pdf/medicamentos/resoluciones/etica_res_8430_1993.pdf.
9. Rabe-Hesketh S, Skrondal A. Multilevel and longitudinal modeling using Stata. Volume II: Categorical responses, counts, and survival. 3rd ed. College Station: Stata Press; 2012.
10. Oviedo HC, Campo-Arias A. Aproximación al uso del coeficiente alfa de Cronbach. Rev Colomb Psiquiatr 2005;34(4):572-80.
11. Campo-Arias A, Herazo E, Oviedo HC. Análisis de factores: fundamentos para la evaluación de instrumentos de medición en salud mental. Rev Colomb Psiquiatr 2012;41(3):659-71.
12. Kraemer HC. Population and sampling. In Evaluating medical test: objetive and quantitative guidelines. Newbury Park: Sage Publications; 1992.Págs.26-62.
13. República de Colombia. Ministerio de Protección Social, Dirección Nacional de Estupefacientes. Estudio Nacional de Consumo de Sustancias Psicoactivas en Colombia 2008. Bogotá: Ministerio de Protección Social; 2008.
14. Castillo M, Campo A, Silva Durán J, Meneses Moreno M, Navarrete Hernández P. Factores asociados con el inicio temprano de relaciones sexuales en estudiates adolescentes de un colegio de Bucaramanga, Colombia. Rev Colomb Psquiatr 2004;33(4):367-77.
15. Rodríguez Rodríguez DC, Dallos Bareño CM, González Rueda SJ, Sánchez Herrera ZM, et al. Asociación entre consumo de alcohol y síntomas depresivos en estudiantes e Bucaramanga, Colombia. Cad Saude Pública 2005; 21(5):109-18.

Urinary levels of early kidney injury molecules in children with vitamin B12 deficiency

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ABSTRACT
The aim of this study was to investigate urine early kidney injury molecules, including human kidney injury molecule-1 (KIM-1), liver-type fatty-acid binding protein (L-FABP), N-acetyl-b-D-glucosaminidase A (NAG), and neutrophil gelatinase-associated lipocalin (NGAL) in children with vitamin B12 (cobalamin) deficiency (CD). Twelve children with vitamin B12 deficiency and 20 healthy matched controls were included. Hematologic parameters, serum urea, creatinine (Cr), electrolytes, B12 and folate levels were recorded. Estimated glomerular filtration rate (eGFR) was calculated. Urine protein, electrolytes, and urinary early markers were measured. Patients with CD had significantly higher urine electrolyte/Cr ratios ($p < 0.05$). Significantly higher urinary KIM-1/Cr, L-FABP/Cr, NAG/Cr and NGAL/Cr were found in CD group ($p < 0.05$). Significant negative correlations were found between levels of serum B12 and urinary markers in the patients ($p < 0.05$). Increased urinary kidney injury molecules and electrolytes in children with B12 deficiency suggest a possible subclinical renal dysfunction, which cannot be determined by conventional kidney function tests.
Key words: *vitamin B12 deficiency, kidney injury, KIM-1, L-FABP, NAG, NGAL.*

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INTRODUCTION

Vitamin B12 (cobalamin) is an essential ingredient for cell growth and proliferation, hematopoiesis, and neurological functions.[1] Cobalamin deficiency (CD) is a worldwide problem in all age groups. Cobalamin deficiency may result in a variety of symptoms, since vitamin B12 is essential for nucleic acid synthesis, erythrocytes and for the maintenance of myelin.[2] Neurological symptoms such as hypotonia, seizures and developmental delay can be seen in infants, while in older children megaloblastic hematological changings, extrapyramidal signs and delirium or psychosis can be observed.[3] Selective vitamin B12 malabsorption or Gräsbeck-Imerslund syndrome has been reported together with proteinuria[4]. In addition, if CD advances, it will cause anemia,[2] which may lead to chronic hypoxia as one of the causes of kidney injury.[5] Although hematological and neurological aspects of CD have been investigated extensively, there is no study on renal effects of CD in the literature search.

Serum levels of blood urea nitrogen or creatinine do not increase until more than half of the kidney functions are lost.[6] Therefore, non-invasive early biomarkers that predict subclinical renal damage are necessary. The most promising biomarkers are human kidney injury molecule-1 (KIM-1), liver-type fatty-acid binding protein (L-FABP), N-acetyl-b-D-glucosaminidase A (NAG), and neutrophil gelatinase-associated lipocalin (NGAL). In this study, we used new injury markers including KIM-1, L-FABP, NAG and NGAL to investigate the possible subclinical effects of CD on kidney functions.

According to our search in English literature, we could not find any study investigating early markers of kidney damage in children with B12 deficiency. Thus, in this study, we aimed to investigate the possibility of subclinical renal injury, which cannot be determined through conventional methods in children with CD.

MATERIALS AND METHODS

Twelve children (7 male, 5 female) with CD, who admitted to the Dicle University Hospital between April and December 2015 period were included. The control group consisted of age- and gender-matched 20 healthy children (11 male, 9 female), who were admitted to the hospital due to a routine check-up or minor surgery, without

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anemia or any disease/drug use affecting the kidney functions. Medical history, complaints, and physical examination findings were recorded. Children with diabetes mellitus, rheumatic disease, urinary tract infection, sepsis, kidney disease, history of nephrotoxic drug use, or liver, heart or respiratory disease were excluded.

Blood hemoglobin (Hb), white blood cell count (WBC), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and platelet count (PLT) were recorded. Anemia in childhood was defined as a hemoglobin (Hb) concentration below cut off levels established by the World Health Organization: 11 g/dl in children aged 6-59 months, 11.5 g/dl in children aged 5-11 years and 12 g/dl in children 12-14 years old.[7] Biochemical analyses included, serum urea, creatinine (Cr), electrolytes [sodium (Na), potassium (K), calcium (Ca)], aspartate transaminase (AST), alanin aminotransferase (ALT), lactate dehydrogenase (LDH), C-reactive protein (CRP), and B12 vitamin and folate levels. Diagnosis of CD was done when vitamin B12 levels were <148 pmol/L, with normal folate levels, and with exclusion of other causes of anemia.[2]

Urine samples of 10 ml were centrifuged for 3 minutes at 3000 rpm and urine at upper part of the tube was filled in four separate Eppendorf tubes and stored at -80 ºC until measurements. The following variables were studied in the urine samples: Ca, Na, K, magnesium (Mg), uric acid (UA), protein, and Cr. Urinary early markers of kidney injury including KIM-1, L-FABP, NAG and NGAL were also measured. Biochemical parameters were investigated by an Abbott ARCHITECT C16000 (Abbott Park, IL) device through an enzymatic colorimetric method. Early markers of kidney damage were examined by enzyme linked immunosorbent assay (ELISA) method. Urine electrolyte and early renal injury markers' concentrations were divided by the urine creatinine for comparisons. The eGFR was estimated from serum creatinine by using bedside Schwartz formula for children.[8]

The study complied with the Declaration of Helsinki, all subjects gave informed consent and the study protocol was approved by the Ethics Committee of the Dicle University Medical School.

Numerical data were presented as mean plus/minus standard deviation, median, and maximum–minimum; whereas categorical data were given as a number and percentage. Mann-Whitney U-test was used for comparison between independent groups. Chi-square test was used to compare the categorical data. Relationships between data were investigated by using Spearman's correlation analyses. P value of less than 0.05 was considered statistically significant.

Table 1. *Demographic and biochemical characteristics of cobalamin (B12) deficiency and the control group [Median (minimum-maximum)]*

	B12 Deficiency group (n= 12)	**Control group (n= 20)**	***p***
Age (years)	8.0 (1.0 - 14.0)	5.0 (2.0 - 11.0)	NS
Height (cm)	125.0 (74.0 - 157.0)	109.0 (75.0 -145.0)	NS
eGFR(ml/min/1.73 m^2)	101.7 (90.6 - 115.8	104.7 (83.4 - 121.5)	NS
Serum vitamin B12 (pg/ml)	111.0 (50.0 - 147.0)	298. 0 (122 - 442.0)	<0.001
Serum folate (ng/ml)	17.5 (9.2 - 23.0)	15.0 (11.0 - 19.0)	NS
Serum glucose (mg/dl)	92.0 (84.0 - 124.0	87.0 (79. 0 - 100.0)	NS
Serum urea (mg/dl)	17.0 (15.0 - 223.0)	18.0 (15.0 - 23.0)	NS
Serum creatinine (mg/dl)	0.50 (0.31 - 0.56)	0.49 (0.33 - 0.58)	NS
Serum sodium (mEq/L)	(136.0 (134.0 - 139.0)	137.0 (131.0 - 141.0)	NS
Serum potassium (mEq/L)	4.5 (3.1 - 5.3)	4.3 (3.8 - 5.9)	NS
Serum calcium (mg/dl)	10. (7.8 - 10.4)	9.7 (9.0 -11.0)	NS
Serum AST (IU/L)	34.0 (15.0 - 42.0)	26.5 (14.0 - 48.0)	NS
Serum ALT (IU/L)	20.0 (9.0 - 43.0)	15.0 (8.0 - 35.0)	NS
Serum LDH (IU/L)	345.0 (223.0 - 2313.0)	254.5 (169.0 - 330.0)	<0.001

eGFR: Estimated glomerular filtration rate; AST: Aspartat transaminase; ALT: Alanin aminotransferase; LDH: Lactate dehydrogenase; NS: Not significant.

RESULTS

Twelve (7 male, 5 female) children with CD were included. The control group consisted of 20 (11male, 9 female) healthy children. There was no significant difference in the mean age of children with CD and the healthy controls (6.9 ± 4.6 vs. 5.3 ± 2.4 years) (p >0.05) (*Table 1*).

No significant differences were found in eGFR, and serum urea, Cr, Na, K, Ca, ALT, AST, glucose, and folate levels between CD patients and the control subjects. The mean serum vitamin B12 level was significantly lower in the CD group compared to the controls (p <0.001); however, serum LDH level was significantly higher in CD group (p <0.001) (*Table 1*).

Significantly lower values of RBC, Hb, Hct (p <0.001) and higher levels of MCV and MCHC were found in CD group (p <0.001; p= 0.035, for each). There were no significant differences in WBC and PLT counts between two groups (p >0.05) (*Table 2*).

Children with CD had significantly higher urine KIM-1/Cr, NGAL/Cr, NAG/Cr and L-FABP/Cr ratios compared with the healthy controls (p= 0.008; p= 0.019; p= 0.003; p= 0.011; respectively) (*Table 3*).

Although CD patients had significantly higher urine Na/Cr, and UA/Cr values compared to the control group (p= 0.029; p= 0.025, respectively), no significant differences were found in urine P/Cr, Mg/Cr,K/Cr, Ca/Cr and Protein/Cr values between the two groups (*Table 4*).

Table 2. *Hematological characteristics of cobalamin deficiency and the controls groups [Median (minimum-maximum)]*

	B12 Deficiency group (n= 12)	**Control group (n= 20)**	***p***
WBC	9.1 (8.8 - 14.4)	8.0 (5.4 -10.4)	NS
RBC (x10^6 cells/µL	3.7 (1.3 - 4.2)	4.8 (4.2 - 6.6)	<0.001
Hemoglobin (g/100 mL)	10.1 (4.4 - 11.2)	13.0 (12.0 - 15.0)	<0.001
Hematocrit (%)	30.4 (12.6 - 33.6)	39.0 (32 - 49.0)	<0.001
MCV (fL)	90.0 (82.3 - 96.0)	80.1 (73.4 - 87.0)	<0.001
MCHC (g/dL)	34.3 (32.1- 36.9)	32.4 (27.0 - 35.0)	0.031
PLT count (×10^3/mm^3)	273.0 (127.0 - 664.0)	288.5 (304.6 - 664.0)	NS

WBC: White blood cells; RBC: Red blood cells; MCV: Mean corpuscular volume, MCHC: Mean corpuscular hemoglobin concentration; PLT: Platelets; NS: Not significant.

Table 3. *Urinary levels of early kidney injury molecule cobalamin deficiency and the control groups [Median (minimum-maximum)]*

	B12 Deficiency group (n= 12)	**Control group (n= 20)**	***p***
KIM-1/Cr	0.08 (0.01 - 0.32)	0.01 (0.00 - 0.03)	0.008
NGAL/Cr	19.5 (3.1 - 87.1)	3.9 (1.1 - 10.5)	0.019
NAG/Cr	0.81 (0.13 - 5.85)	0.19 (0.04 - 0.70)	0.003
L-FABP/Cr	3.87 (0.76 - 19.21)	0.90 (0.34 - 2.09)	0.011

NGAL: Neutrophil gelatinase-associated lipocaline; NAG: N-acetyl-b- D -glucosaminidase; L-FABP: Liver-type fatty acid-binding protein;KIM-1: Human Kidney injury molecule-1; Cr: Creatinine.

Table 4. *Urinary electrolytes to creatinine ratios in the cobalamin deficiency and control group [Median (minimum-maximum)]*

	B12 Deficiency group (n= 12)	**Control group (n= 20)**	***p***
Na/Cr	2.27 (1.21 - 14.25)	1.74 (0.40 - 5.37)	0.029
K/Cr	0.76 (0.39 - 2.33)	0.56 (0.19 - 2.25)	NS
Ca/Cr	0.12 (0.02 - 1.30)	0.05 (0.01 - 0.26	NS
P/Cr	0.52 (0.15 - 2.02)	0.65 (0.23 - 1.22)	NS
Mg/Cr	0.17 (0.05 - 0.25)	0.09 (0.03 - 0.25)	NS
Protein/Cr	0.09 (0.05 - 0.26)	0.09 (0.03 - 0.21)	NS
Uric acid/Cr	0.93 (0.07 - 2.21)	0.22 (0.04 - 1.56)	0.025

Na: Sodium; K: Potassium; Ca: Calcium; P: Phosphorus; Mg: Magnesium; Cr: Creatinine.

Significant positive correlations were found between urine KIM-1/Cr, L-FABP/Cr, NAG/Cr and NGAL/Cr ratios in the CD group ($p < 0.05$). There were significant negative correlations between serum vitamin B12 level and urine biomarker/Cr ratios ($p < 0.05$). In addition, significant negative correlations were found between values of Hb and Hct and urine biomarkers'ratios ($p < 0.05$). Finally, there were significant negative correlations between RBC and ratios of urine L-FABP/Cr and KIM-1/Cr in CD patients (*Table 5*).

DISCUSSION

Increased urinary Na/Cr and UA/Cr in our CD children may be a sign of tubular dysfunction. In chronic renal failure, urinary injury biomarkers were found to be decreased if low hemoglobin levels corrected by the administration of erythropoietin.[9] Anemia occurring in CD deficiency may lead to chronic hypoxia. Renal tubular cells had a high metabolic activity and oxygen consumption and highly susceptible to hypoxia.[10]

KIM-1, as a transmembrane protein, is upregulated in proximal tubular cells following ischemic or nephrotoxic injury and is expressed in the fibrotic areas of the damaged kidneys.[11] We found increased urinary KIM-1 levels in children with CD as a marker of tubulointerstitial changes.

L-FABP is thought to be an endogenous antioxidant that suppresses tubulointerstitial injury and is a biomarker of chronic renal damage and its progression.[12] Therefore, urinary L-FABP could be a novel biomarker for chronic intrarenal ischemia. Higher urinary L-FABP excretion was found in our CD patients may be a sign of chronic hypoxemia resulted from CD anemia.

NAG is an abundant lysosomal enzyme existing in the renal tubular cells and released in the urine during proximal tubular injury.[13] NGAL is a member of the lipocalin family, and has been identified as a sensitive biomarker of renal tubular damage in acute and chronic nephropathy.[14] Significantly increased urinary NAG and NGAL excretion in our CD patients may be indicative of subclinical injury of renal tubules.

Damage in kidney tubular cells can lead to tubular dysfunction with impaired electrolytes reabsorption. In our study, increased urine excretion of Na and uric acid were found. Moreover, increased urine kidney injury molecules of our patient suggested kidney damage. Correlation of these four novel biomarkers indicated that these patients may have kidney damage and these markers are valuable.

Although there was no difference in urine protein/Cr ratios between our CD patients and healthy subjects, Wahlstedt-Fröberg et al., reported elevated excretion of protein in their six patients with Gräsbeck-Imerslund syndrome. Non-elevated urine protein of our patients may be related to different etiopathogenesis of our

TABLE 5. *Spearman's correlation coefficients between urinary novel biomarkers and other variables in cobalamin deficiency group*

		NGAL/Cr	NAG/Cr	L-FABP/Cr	KIM-1/Cr
B12	r	-0.396	-0.475	-0.453	-0.458
	p	0.005	0.001	0.001	0.001
Hb	r	-0.325	-0.365	-0.349	-0.360
	p	0.026	0.012	0.016	0.013
RBC	r	-0.322	-0.297	-0.339	-0.358
	p	0.027	0.043	0.020	0.014
MCV	r	0.281	0.418	0.364	0.340
	p	0.049	0.003	0.010	0.017
NGAL/Cr	r		0.931	0.969	0.977
	p		<0.001	<0.001	<0.001
NAG/Cr	r			0.958	0.945
	p			<0.001	<0.001
L-FABP/Cr	r				0.984
	p				<0.001

Hb: Hemoglobin; RBC: Red blood cells; MCV: Mean corpuscular volume;NGAL: Neutrophil gelatinase-associated lipocalin; NAG: N-acetyl-b- D -glucosaminidase; L-FABP: Liver-type fatty acid-binding protein.

patients (dietary CD) contrast to selective B12 malabsorption of Gräsbeck-Imerslund syndrome.[4]

Negative correlations between urinary kidney injury molecules and Hb and vitamin B12 levels in the CD deficient group, supports the existence of a chronic process that renal injury increases parallel to deepening of CD.

The main limitation is the small sample size of CD group, and the other limitation is the cross-sectional design of the study. We could not measure urinary biomarkers after completion of cobalamin supplementation due to fall down of most patients from follow up.

In conclusion, despite normal levels of commonly used conventional kidney function tests, increased urine kidney injury molecule levels may indicate the existence of subclinical renal damage in patients with vitamin B12 deficiency. ■

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REFERENCES

1. Briani C, Dalla Torre C, Citton V, Manara R, et al. Cobalamin deficiency: clinical picture and radiological findings. *Nutrients* 2013;5(11):4521-39.
2. Pawlak R, Lester SE, Babatunde T. The prevalence of cobalamin deficiency among vegetarians assessed by serum vitamin B12: a review of literature. *Eur J Clin Nutr* 2014;68(5):541-8.
3. Whitehead VM. Acquired and inherited disorders of cobalamin and folate in children. *Br J Haematol* 2006; 134(2):125-36.
4. Wahlstedt-Fröberg V, Pettersson T, Aminoff M, Dugué B, et al. Proteinuria in cubilin-deficient patients with selective vitamin B12 malabsorption. *Pediatr Nephrol* 2003;18(5): 417-21.
5. Ozcay F, Derbent M, Aldemir D, Turkoglu S, et al. Effect of iron deficiency anemia on renal tubular function in childhood. *Pediatr Nephrol* 2003;18(3):254-6.
6. Anderson RJ, Barry DW. Clinical and laboratory diagnosis of acute renal failure. *Best Pract Res Clin Anaesthesiol* 2004; 18(1):1-20.
7. Scott SP, Chen-Edinboro LP, Caulfield LE, Murray-Kolb LE. The impact of anemia on child mortality: an updated review. *Nutrients* 2014;6(12):5915-32.
8. Schwartz GJ, Work DF. Measurement and estimation of GFR in children and adolescents. *Clin J Am Soc Nephrol* 2009;4(11):1832-43.
9. Nakamura T, Sugaya T, Kawagoe Y, Suzuki T, et al. Effect of erythropoietin on urinary liver-type fatty-acid-binding protein in patients with chronic renal failure and anemia. *Am J Nephrol* 2006;26(3):276-80.
10. Nangaku M. Chronic hypoxia and tubulointerstitial injury: a final common pathway to end-stage renal failure. *J Am Soc Nephrol* 2006;17(1):17-25.
11. Lim AI, Tang SC, Lai KN, Leung JC. Kidney injury molecule-1: more than just an injury marker of tubular epithelial cells? *J Cell Physiol* 2013;228(5):917-24.
12. von Eynatten M, Baumann M, Heemann U, Zdunek D, et al. Urinary L-FABP and anaemia: distinct roles of urinary markers in type 2 diabetes. *Eur J Clin Invest* 2010;40(2): 95-102.
13. Martensson J, Martling CR, Bell M. Novel biomarkers of acute kidney injury and failure: clinical applicability. *Br J Anaesth* 2012;109(6):843-50.
14. Devarajan P. Review: neutrophil gelatinase-associated lipocalin: a troponin-like biomarker for human acute kidney injury. *Nephrology (Carlton)* 2010;15(4):419-28.